Observations on the Shape of the Nucleus and its Determination.

By

Christian Champy,

Professeur agrégé à la Faculté de Médecine de Paris,

and

H. M. Carleton,

Demonstrator in Histology, University of Oxford.

With Plates 23 and 24 and 11 Text-figures.

Contents.

1. Introductory.

That the nucleus is extraordinarily variable in shape, not only in different animal cells but also in the same cell during the different phases of its ontogeny and metabolism, is a notorious fact. In the following notes, which embody a brief description

of nuclear shape, we have also attempted to analyse, when possible, the factors responsible for this.

In the present stage of cytology the interpretation of cell-function is largely based on purely descriptive methods. Therefore such reasons as we have been able to put forward in explanation of the diversity of nuclear shape are to be regarded more as reasonable suppositions than as proven statements. We are of opinion that it is better to run the risk of assigning false causes to the phenomena which we have observed, than to explain nothing by confining ourselves to purely morphological considerations.

Only when cytology has acquired experimental methods will it be possible rigorously to determine the factors responsible for nuclear form and function.

Although the details of the structure of the nucleus—and particularly those concerning the disposition of the chromatin and the alleged ' linin ' network—are controversial, observations on nuclear shape are easily verified. For not only are the appearances similar with widely different methods of fixation and staining, but they can be controlled by observations on living material. And, finally, corroborative evidence can sometimes be obtained by experimental methods such as tissue culture.

2. The Relation between Nuclear Shape and Surface Tension.

A spherical nucleus is found in hepatic and most other gland-cells, also in many nerve-cells and spermatocytes. Its shape may often be attributed to surface tension, being the result of a relatively fluid (nuclear) mass that is immiscible in the surrounding cytoplasm. Such nuclei are relatively rare in the animal body, for the spheroidal condition is not uncommonly associated with mechanical factors, e.g. furrows or canaliculi in the nuclear membrane. Such structures, which occur more often than is generally supposed, sometimes make it difficult to say whether a spherical nucleus is the result of surface tension alone or of accompanying mechanical causes.

It is a curious fact that such nuclei usually contain a single nucleolus only, and that this body tends to be in the centre of the nucleus or somewhat deviated towards that pole of it which is farthest from the centrosome. The latter seems to exercise a repellant action on the nucleus also—a fact which can be verified in many cells with large nuclei, e.g. spermatocytes.

Lobulation of the nucleus can sometimes be attributed to variations in surface tension at the interfaces of

TEXT-FIG. 1.
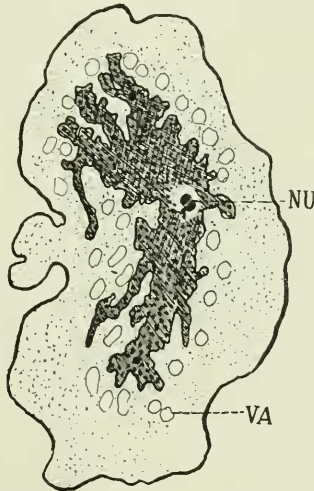
Phagocytic cell (amoebocyte) from a larva of Phryganea sp.— a caddis fly. Extreme polymorphism of the nucleus probably due to variations in surface tension over the nuclear membrane. NU, nucleolus; VA, vacuole. Technique: Bouin and iron haematoxylin.

nucleus and cytoplasm. A striking example of this is furnished by the large cells accompanying histolysis during metamorphosis in insects. Here, as is well known, the larval tissues are destroyed by large phagocytic cells known as Amoebocytes. Text-fig. 1 shows such an element from a larva of Phryganea sp. Here the polymorphism of the nucleus is extreme, while the nucleolus, which is single and central, does not appear to be involved in the lobulation. Of the latter, every degree

may be observed in such cells, and it seems definitely to be related to variations in surface tension caused by exchanges between nucleus and cytoplasm, as has been suggested by various authors (e.g. Prenant, **10**).

In other instances, however, the shape of the nucleus, notwithstanding its extreme lobulation, is too definite to permit of its being attributed to surface tension alone. Examples of this are the spermatogonia of some Amphibia, in which the shape of the nucleus is constant in a given species (Pl. 23, fig. 3). Here the nuclear polymorphism is apparently due to the intervention of other factors (to be considered later), and only such variations from the normal as occur during periods of intensive cell-activity—such as growth, differentiation, &c.— can be ascribed to the surface-tension changes that accompany such phenomena. Somewhat similar are the modifications which occur in many oocytes during development, as shown in Pl. 23, fig. 2. In the early stages of differentiation the nucleus in such elements is oval, containing one large central nucleolus and many smaller peripheral ones. But subsequently the nucleus becomes polymorphic, while around it is established a clear (endoplasmic) zone in the cytoplasm. Here again do we find extreme nuclear lobulation coinciding with enhanced metabolism of the cell.

3. Mechanical Deformation of the Nucleus.

The study of our material has convinced us that nuclear shape is often due to pressure exerted on it by various cell inclusions. An obvious example of this is furnished by the thin and crescentic nucleus entirely pressed to the periphery of the fully developed adipose cell. Somewhat similar is the deformation of the nucleus in the duct-cells from the pronephros of Triton (see Pl. 23, fig. 4). This is due to the centre of the cell being occupied by the lumen of the duct. Again, in the early segmentation stages of ova containing much yolk, the nuclei are indented by the large, inert yolk-discs. Text-fig. 6 shows such a nucleus from a blastula of the Amphibian Triton alpestris. On one side there are deep indentations

between the centrosome and the nuclear membrane : these
are due to other causes and will be referred to later.   And,
finally, similar appearances can be seen in the nuclei of the
interstitial cells of the testicle of R a n a   e s c u l e n t a, the
nuclear membrane here being pitted by the lecithin globules
in the cytoplasm.

Sometimes the inclusions are localized in a particular area
of the cytoplasm. This may give rise to a peculiar deformation
of the nucleus such as is depicted in Pl. 23, fig. 6, which illustrates
a cell from the hepato-pancreas of the isopod crustacean
O n i s c u s.   Here the nucleus at the basal, i.e. attached,

TEXT-FIG. 2.
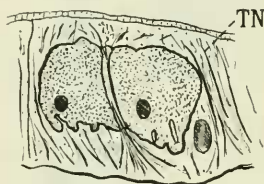
Cell from pronephros of a 3 mm. larva of T r i t o n   a l p e s t r i s.
Note constriction of middle of nucleus due to pressure from
Tonofibrillae, TN.

pole of the cell is strikingly indented by large cytoplasmic
globules of a lipoid nature. It follows from this that nuclear
deformation can be produced by relatively fluid bodies.

Another instance of nuclear shape being modified by cyto-
plasmic structures is afforded by the intestinal epithelial cells
of the same species.   By appropriate staining methods (see
Pl. 24, fig. 3) fine fibrils lying in the cytoplasm around the
nucleus can be distinguished. They run from the basement
membrane to the cuticle, apparently function as an intra-
cellular skeleton, and may be termed T o n o f i b r i l l a e after
the French ' Tonofibrilles '.

We have also observed a similar condition in cells from the
excretory tubules of larvae of T r i t o n as shown in Text-
fig. 2.

In those muscles which are characterized by cross-striation

of their fibres, i.e. ordinary striated and cardiac muscle, the
influence of cytoplasmic structures on nuclear shape is very
marked. Thus, in striated muscle there is obvious flattening
of the nuclei against the sarcolemma due to pressure from the
areas of Cohnheim (i.e. groups of fibrils) of which the fibre is
composed.

Often, however, other causes intervene, chief amongst which
is the influence of the Membrane of Krause ('Strie Z'
of the French and 'Zwischenscheibe' of the German authors).

TEXT-FIG. 3.
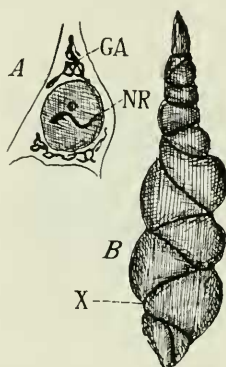
A, After Cajal, showing intranuclear rodlet, NR. in pyramidal cell
from cerebral cortex of rabbit. Technique : Cajal method for
Golgi apparatus, GA.   B, After Retzius, depicting peri-nuclear
structure (x) in spermatozoon of the Gasteropod Cypraea.

This structure is segmentally disposed along the muscle fibril
and appears in the middle of the dark bands as a clear and
narrow line. It is best studied in the large fibres of insects
(see Text-fig. 10), where it can be seen to constrict the nucleus
at regular intervals by its projection out of the fibrils into the
surrounding sarcoplasm.

A similar appearance of the nuclei can be seen in human
cardiac and other vertebrate muscle. This is shown in Pl. 23,
fig. 7. But here we have not been able to follow the membrane
of Krause as far as the nuclear membrane. Nevertheless, the
nuclei bear definite constrictions corresponding to the mem-
branes of Krause of adjacent fibrils, while the curious blunt-

ended nuclei—so characteristic of human heart muscle—can only be explained by assuming the presence of these membranes lying invisible in the sarcoplasm at each end of the nucleus.

TEXT-FIG. 4.
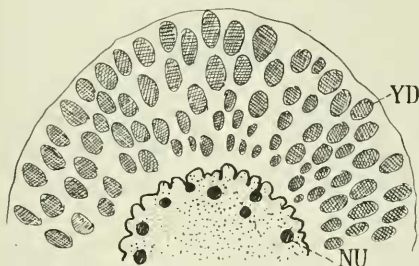
Oocyte of Esox lucius—a pike. Note the pouches in nuclear membrane usually in relation with the nucleoli. NU, nucleolus ; YD, yolk-discs. Technique : Bouin and iron haematoxylin.

4. NUCLEAR SHAPE AND THE CENTROSOME.

We deliberately confine ourselves to the consideration of the centrosome and nucleus in the resting cell, as the question of the spindle fibres, amphiaster and chromosome formation is beyond the scope of these observations. In the resting cell the centrosome often lies very close to the nuclear membrane and opposite an indentation in it. And since this body often does not touch the nucleus, one must surmise that the depression is due not to mechanical causes but to repulsion between nuclear membrane and centrosome. When an amphiaster is present, its influence upon the nucleus is still more marked, as is shown in Text-fig. 6, which depicts a blastomere from an egg of Triton. It will be seen that here nuclear shape is due partly to pressure from the yolk-discs (as already pointed out), partly to invaginations in the nuclear membrane in the vicinity of the centrosome. The astral rays in fact deeply indent the nucleus wherever they come into contact with it—a point possibly in favour of the view that the cytoplasmic radiations around the centrosome are of a relatively solid nature.

5. THE RELATION BETWEEN CELL SHAPE AND NUCLEAR SHAPE.

It is notorious that the longer a cell, the longer (usually) is its nucleus. Muscle, columnar epithelium, and connective-tissue cells are familiar examples of this (see Pl. 23, figs. 1, 8, and 10 ; Pl. 24, fig. 2). This elongation of the nucleus is often due to mechanical causes. Thus, in epithelia it is sometimes due to mutual cell-pressure, while the long nucleus of the smooth muscle-fibre must be ascribed to pressure from the myofibrillae. Further, the nucleus shortens or lengthens as the fibre contracts or extends. Again, in preparations of amphibian intestine fixed in different degrees of distension, there are marked differences in the height of the epithelial cells and their nuclei—the two varying in length in a parallel ratio between certain limits. Exceptions, however, exist to this general rule. For instance, in the intestinal epithelial cells of the dragon-fly Libellula (see Pl. 24, fig. 5) the small oval nucleus is quite disproportionate to the elongated cell.

As claimed by Martin Heidenhain (**8**), we must surmise the existence of a force which tends to push the nucleus towards the centre of the cell. And in view of the plasticity of the nucleus there can be no doubt but that this force must influence its shape also.

It is a fact of no small significance that the nucleus never comes into contact with the cell membrane, except in a few instances due to powerful mechanical factors, e.g. pressure from bulky cytoplasmic inclusions forcing the nucleus against the cell membrane. Two possibilities suggest themselves in explanation of this :

(1) That the position of the nucleus is due to forces exerted on it by the surrounding cytoplasm, forces which might conceivably be proportional to the mass of the cytoplasm around the nucleus. Were this so, nuclear shape in a cell of greater length than breadth would

be as in Text-fig. 9, B on p. 600, which is never the case
in nature.

(2) That there is mutual repulsion between
cell membrane and nuclear membrane. Such a
force, acting in an inverse ratio to the distances between the
two membranes is indicated in Diagram C, p. 600. This
supposition explains :

(a) Why nuclear and cell membranes practically never come
into contact with one another.

(b) Why the nucleus tends to elongate concurrently with
the cytoplasm.

(c) Why the nucleus is never round so long as the length of
a cell is greater than its breadth, although there is often ample
room in the cytoplasm for it to become spherical.

Of the nature of such a force responsible for the antagonism
between cell membrane and nuclear membrane we know
nothing.

6. Canaliculi in the Nuclear Membrane.

Intranuclear canaliculi are more common in spherical and
oval nuclei than is usually thought. They have been described
in the spermatogonia of Amphibia by Champy (4), and are
easy to demonstrate in Rana esculenta and the Axolotl.
Canaliculi in the nuclear membrane occur in many types of
cell ; we have observed them in the epithelium lining the
Wolffian duct in the salamander, and in pyramidal cells of
the cerebral cortex in the guinea-pig. These structures are
illustrated in Pl. 24, fig. 4, and in Text-fig. 8.

The intranuclear canaliculus is essentially a narrow invagina-
tion of the nuclear membrane. Its blind extremity, which
may be bifid, often ends in the vicinity of the nucleolus. That
this structure is a definite tube and not a deep furrow in the
nuclear membrane, is shown in transverse sections of it. In
many spermatogonia there seems to be some relation between
the canaliculus and the centrosome ; at the prophase the latter
comes to lie very close to the former, often exactly opposite
its aperture in the nuclear membrane.

TEXT-FIG. 5.
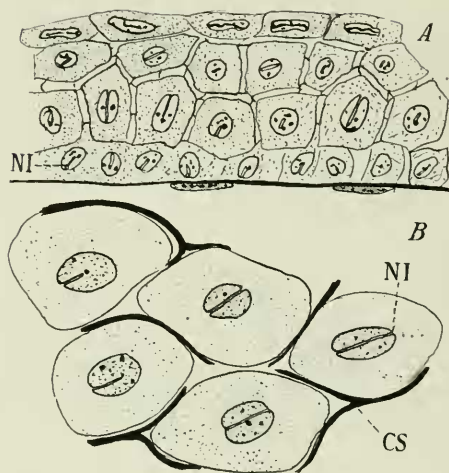
A, Skin of sucker of Lepadogaster guannii--a 'suck-fish'.
B, Supporting tissue of the same organ with intercellular
stroma of cartilage. Both A and B show intranuclear canaliculi
in all the cells. Probably mechanical in origin, e. g. mutual cell-
pressure.   CS, Intercellular cartilaginous stroma; NI, Nuclear
incision.

TEXT-FIG. 6.
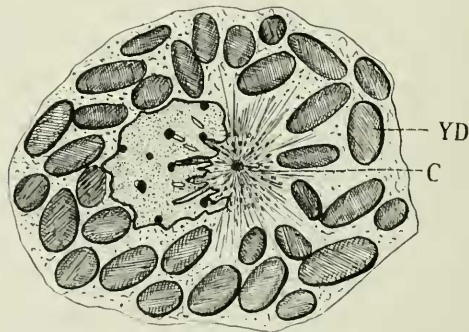
Blastomere from blastula of Triton alpestris. Note deforma-
tion on one side by yolk-discs, YD, and on the other by astral rays.
C, Centrosome. Technique : Champy's fluid and iron haematoxylin.

The intranuclear canaliculus of nerve-cells (see Text-figs. 7 and 8) is sometimes demonstrated by the Cajal method for the Golgi apparatus, and has apparently been observed by Cajal himself. With standard cytological stains—such as iron haematoxylin—it appears as a single invagination of the nuclear membrane. Its aperture is often opposite the point

TEXT-FIG. 7.
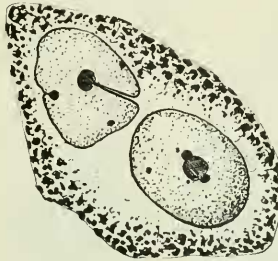
Binucleated sympathetic ganglion cell from rabbit.
Intranuclear canal in one of nuclei.

TEXT-FIG. 8.
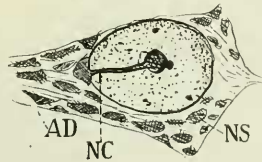
Pyramidal cell from cerebral cortex of guinea-pig.
AD, Apical dendrite; NC, Intranuclear canal; NS, Nissl substance.

of insertion of the apical dendrite in the case of pyramidal cells (see Text-fig. 8). In these elements the relation of the canaliculus to the centrosome is obscure, largely owing to the uncertainty of the existence of this structure in adult nerve-cells.

Intranuclear canaliculi are also readily observed in the cells lining the Wolffian ducts in Amphibia, while apparently similar structures can sometimes be seen in the tissues of the higher Vertebrates, though here, except in the case mentioned above, the small size of the cells renders observation difficult.

7. Folds and Incisions in the Nuclear Membrane.

Such modifications of the nucleus are common, though care
is required in their observation. This is easiest after fixation
in fluids which do not precipitate the nuclear contents in too
coarse a manner. Fixatives such as Gatenby's Flemming
without acetic (**6**) and Champy's carbol-formalin (**4**) give the
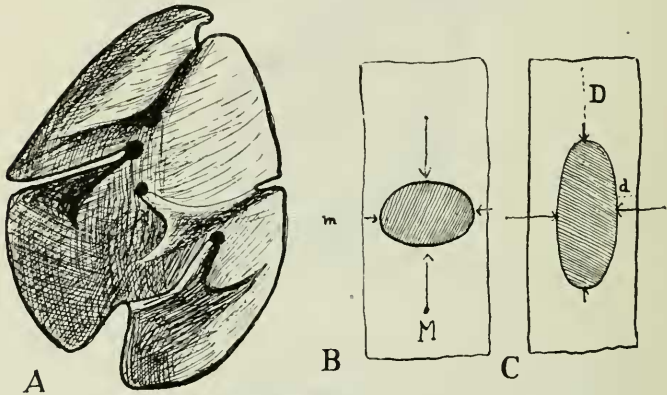best results.

TEXT-FIG. 9.

Fig. A.—Diagram showing the relation of intranuclear incisions
to the nuclear membrane and nucleoli.

Figs. B and C showing that the shape of the nucleus is governed
rather by its distance from the cell membrane than by any mass
action of the cytoplasm. Were nuclear shape the product of
repulsion between the nuclear membrane and the mass of the
cytoplasm, the shape of the nucleus in an elongated cell would
be as shown in B. But this is never the case. In nature the
long axis of the nucleus is always in the long axis of the cell,
as indicated in C. The explanation of this seems to be that the
nuclear membrane is repelled by the cell membrane, and that
the nearer it is to the latter, the greater the degree of repulsion.

Gastric epithelium of Amphibia, e.g. of B o m b i n a t o r or
A l y t e s , in which the cells are very large, shows clearly the
folds in the nuclear membrane. In longitudinal sections of the
nuclei there may be several of these structures, which may or
may not traverse its entire length. They are illustrated in
Pl. 23, figs. 1, 7 A, and 8. That we are dealing with folds and not
with canaliculi is made clear by transverse sections of such
nuclei, which are depicted in Pl. 23, figs. 7 B and C, and 8.

Folds in the nuclear membrane are found in a great variety of cells in addition to gastric epithelium in Amphibia. They occur in cardiac muscle in Man and A s t a c u s (the crayfish), and also in the connective-tissue cells of the Testis in the latter species. In germinal epithelium they are especially common, not only in that of the A x o l o t l (Pl. 23, fig. 10) but also in some mammalian tissues. But in the latter it is usually difficult to make sure that the structures one can see

TEXT-FIG. 10.
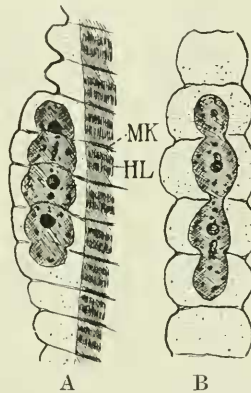
Portions of muscle-fibres from nymph of P h r y g a n a e a s p.— a caddis fly. In A the membranes of Krause can be seen running across the sarcoplasm and constricting the nucleus at regular intervals. In B only the nuclear constrictions are visible, the section passing outside the zone of myofibrillae. HL, Hensen's line; MK, Membrane of Krause.

in germinal epithelium are truly intranuclear folds, though it is interesting to note that undoubted incisions exist in the pathological cysts—C y s t a d e n o m a t a—which are derived from this epithelium.

The nuclei of smooth muscle-fibres, after impregnation by the Cajal method for the Golgi-apparatus (Cajal, **1** ; Carleton, **2**), show a peculiar spiral peri-nuclear band which has been observed by Rio Hortega (**11**). After careful differentiation, iron haematoxylin sections show that this structure is not a thickened portion of the nuclear membrane but a series of usually rather irregularly arranged spiral folds. Transverse

sections of such nuclei confirm the existence of these incisions, which we have observed in non-striated muscle from the intestine in Amphibia (see Pl. 24, fig. 2), in Mammals (muscle layers of intestine of cat), and in certain invertebrate muscle-fibres, e.g. heart of Helix as shown in Pl. 24, fig. 6.

Finally, we have noted similar folds in the nuclear membrane of developing oocytes (already described in Section 3), while a peri-nuclear reticulum—possibly comparable to that found in smooth muscle-cells—has been described by Retzius in the spermatozoa of certain Gasteropods as shown in Text-fig. 3, B.

8. The unfolding of Invaginations in the Nuclear Membrane.

It seems certain that nuclear folds and incisions expand under certain conditions, thus altering both volume and shape of the nucleus. That such a phenomenon occurs during differentiation of some cells is shown by the following example :

In Urodele Amphibia there exists a layer of lymphoid tissue surrounding the liver. Study of the lymphocytes in this layer (see Pl. 23, fig. 5) show that their nuclei, though round or oval, bear a large number of narrow incisions. The latter can be observed in various degrees of ' deployment ' in these cells, and there is no doubt that polymorphonuclear leucocytes can be formed in this manner from lymphocytes in some Amphibia —a point in favour of the ' Unicist ' theory of blood-formation. The persistence of some of the nuclear folds gives rise to the lobulation characteristic of the polymorphonuclear leucocyte.

Mutual cell-pressure may apparently in certain cases inhibit expansion of the nuclear membrane. We have observed an instance attributable to this in cells from the epidermal and sub-epidermal tissues of the sucker of the fish Lepado-gaster guannii. This is illustrated in Text-fig. 5, A and B.

9. Intranuclear Rodlets, etc.

Intranuclear rodlets and allied structures, which are only found in highly specialized cells such as spermatids or certain red blood corpuscles, are responsible for the shape of the

nucleus in such elements. The peculiar shape of the head of
the spermatozoon is doubtless an adaptation enabling it
rapidly to move in fluids and to penetrate the ovum.   In
some instances, which have been described by Champy (**4**),
the changes in the shape of the nucleus during the stages
termed 'Spermateleosis' by Gatenby (**7**) are due to the
influence of a special intranuclear apparatus.
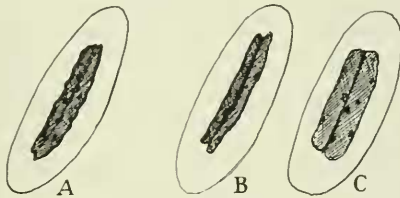
TEXT-FIG. 11.

Fig. A.—Normal red blood corpuscle of bird with intranuclear
   rodlet faintly indicated.
Figs. B and C.—Avian red cells after four days' culture (pigeon's red
   cells in chicken plasma). The nuclei have become swollen and the
   chromatin reduced in amount ; consequently the intranuclear
   rodlet is clearly visible.

The latter is best studied in Amphibia such as B o m -
b i n a t o r, the Salamander, and the Axolotl.   In these it
can be seen within the spermatid as a thin and usually
refringent rod, lying in the long axis of the nucleus. It appears
to be developed from the centrosomes, originating from either
the posterior or the anterior of these structures. Or sometimes
it may be developed from both simultaneously. When the
intranuclear rodlet does not extend the whole length of the
nucleus, its free extremity, which may be bifid, is sometimes
in relation with the nucleolus. All this is indicated in Pl. 24,
fig. 1, which depicts spermatid nuclei from B o m b i n a t o r.
That this structure is not a fold in the nuclear membrane
is seen in the figures of transverse sections of these nuclei.
But it often co-exists with intranuclear canaliculi, from
which, however, it can be further distinguished by its greater
refringency.

It is well known that the red blood corpuscles of birds have

oval nuclei of a very definite aspect. These nuclei are remarkably stable, for they often retain their shape after the rest of the corpuscle has been haemolysed. Now, observation of the normal Avian red cell reveals little beyond a rather dark central portion and, often, a small invagination at both poles of the nuclear membrane. The general appearance is such (see Text-fig. 11) as to suggest the presence of some supporting structure within the nucleus, though the density of the chromatin makes its observation difficult. But when a bird's red blood corpuscles are aseptically cultivated in their own plasma, the nucleus slowly swells up before actual death of the cell occurs. As the nucleus becomes spherical, the chromatin becomes condensed into a single nucleolus, and an axial rodlet can frequently be seen under such conditions.

10. The Relation between Nucleoli and Nuclear Shape.

The nucleolus remains one of the most enigmatical of the cell components, in spite of the attention devoted to it by many biologists, and by Montgomery (**9**) and Vigier (**12**) in particular. The nucleolus is of interest in that it often shows amoeboid movements and undergoes independent fission during the life of the cell. In these observations the term 'nucleolus' is used in its widest sense, as signifying any condensation of nuclear material within the nucleus. Consequently, the word as employed in this paper applies to both karyosomes (or chromatin nucleoli) and plasmosomes (i.e. condensations of the oxyphil substance called plastin). Not only do both chromatin and plastin often occur within the same nucleolus, but karyosomes or plasmosomes sometimes contain one or more granules of unknown composition, which have been shown by the aid of special impregnation methods to divide by fission during mitosis (Carleton, **3**).

Clearly the nucleolus is often a complex structure of doubtful significance, and it is impossible at present to dogmatize on the relation of this element to nuclear shape. At the most, certain deceptive appearances may be cleared up.

Nuclear polymorphism is often—though by no means always—associated with the presence of multiple nucleoli as shown in Pl. 23, figs. 3 and 5. In elongated nuclei the nucleoli usually lie parallel to the main axis of the nucleus as depicted in Pl. 24, figs. 2 and 6, and in Text-fig. 10.

But it is in developing oocytes that the relation between nucleoli and nucleus is particularly deceptive. In the earlier stages of development the nucleoli come to lie at the periphery of the nucleus, and when invaginations subsequently appear in the nuclear membrane, they do so opposite the nucleoli. Pl. 23, fig. 2, and Text-fig. 4 illustrate this, and they suggest the possibility of nuclear incisions being formed under the influence of the nucleoli. On the other hand, it must be observed that in the case of some nuclei, the indentations in which are obviously due to certain of the mechanical causes already considered, the nucleoli are yet often in relation to the blind ends of the pouches in the nuclear membrane. In muscle, too, infolded portions of the latter often come into contact with the nucleoli, though here again nuclear incisions are primarily mechanical in origin. And finally, there are cells the nuclei of which contain nucleoli and yet have a nuclear membrane of regular contour, as shown in Pl. 23, fig. 11.

The main outcome of all this is that the relations so often seen between nucleoli and nuclear invaginations are usually secondary, and that the position of the nucleoli in such instances is rather an effect than a cause.

11. Cell Division and Nuclear Differentiation.

It is not without significance that mitoses are extremely rare—if not altogether absent—in cells the nuclei of which contain well-developed canaliculi or incisions. Such, at any rate, is the case with the following tissues in adult mammals :

Non-striated muscle.

The various segments of the urinary tubule in the kidney.

The epithelium lining the vesicles of the thyroid gland.

Nerve-cells.

Our observations suggest that while highly developed nuclear

canaliculi or incisions seem to be incompatible with mitosis, direct division may occur in cells—other than those enumerated above—which contain such structures. Thus, amitosis has been observed in nuclei of the cells of the Wolffian ducts and germinal epithelium and Sertoli cells ; also possibly in the gastric mucosa of some animals.

The behaviour of smooth muscle when cultured in plasma confirms this idea. It has been shown (Champy, **5**) that the nuclei of this tissue, when removed from the inhibitory influences of the organism, multiply actively. At first they do so amitotically, and only when the typical structure of these nuclei has disappeared by a progressive ' de-differentiation ' do they multiply by mitosis. Cultures of ovarian germinal epithelium behave in a similar manner. Again, the fundus glands of the human uterine mucosa have nuclei without incisions, while the cervical glands possess them. The former divide mitotically, the latter amitotically. And further, even in Adenomata (i. e. benign tumours) derived from the cervical glands does direct division persist. Only when such growths become carcinomatous do mitoses appear.

We would here point out that incisions or lobulations of nuclei have only too often been mistaken as evidence of direct division. In our experience such appearances are only of value when an actual increase of the number of nuclei can be established.

In conclusion, then, there is evidence that well-developed intranuclear canaliculi and incisions are incompatible with mitosis, a fact which possibly explains the tendency towards direct division in certain cells with specialized nuclei.

12. SUMMARY.

Variations in the shape of the nucleus have been described in different animal cells. In addition, the following factors have been shown to be responsible for nuclear shape :

(1) S u r f a c e   t e n s i o n : when this is equal over the surface of the nuclear membrane, the nucleus tends towards the spherical condition. When surface tension varies over the interface

between nucleus and cytoplasm, nuclear polymorphism may result.

(2) Mechanical deformation of the nucleus is common and may be due to various causes, chief amongst which are: (a) Pressure from cytoplasmic inclusions, e.g. fat, lecithin, and yolk; (b) Tonofibrillae; (c) in striated muscle, the influence of the Membranes of Krause which constrict the nucleus along its length—and limit its ends—by their prolongation from the myofibrillae into the sarcoplasm.

(3) The centrosome, which has been shown (in the resting cell) often to repel that part of the nuclear membrane which is nearest to it.

(4) The relation between cell shape and nuclear shape has been briefly discussed. It has been noted that the nucleus never comes into contact with the cell membrane, except in the rarest instances due to the intervention of mechanical factors. Evidence has been brought forward in favour of our view that there is a mutual repulsion between cell membrane and nuclear membrane.

(5) Canaliculi and incisions in the nuclear membrane have been described in various cells.

(6) The unfolding of such incisions during development and differentiation of some such cells has been described.

(7) Intranuclear rodlets and their importance in the maintenance or the modifying of nuclear shape have been discussed.

(8) Mitotic division and a certain degree of nuclear differentiation have been shown often to be incompatible—thereby accounting for amitosis in certain highly specialized nuclei.

(9) The need for care in distinguishing between nuclear incisions and genuine amitotic division of nuclei has been emphasized.

June 1921.

13. Bibliography.

1. Cajal.—" Algunas variaciones fisiológicas y patológicas del aparato reticolar de Golgi ", ' Trab. del Lab. Invest. Biol. Madrid ', vol. 10, 1912.

2. Carleton.—" Note on Cajal's Formalin-Silver nitrate method for the Golgi-apparatus ", ' Journ. Roy. Micr. Soc.', 1919.

3. —— " Observations on an intranucleolar body in columnar epithelium cells of the Intestine ", ' Quart. Journ. Micr. Sci.', vol. 64, 1920.

4. Champy.—" Spermatogenèse des Batraciens ", ' Arch. de Zool. Exp.', vol. 52, 1913.

5. —— " Résultats de la méthode de culture des tissus en dehors de l'organisme ", ' Presse médicale ', no. 9, 1914.

6. Gatenby.—" Cytoplasmic Inclusions of the Germ Cells ", Pt. I, ' Quart. Journ. Micr. Sci.', vol. 62, 1916-17.

7. —— " Cytoplasmic Inclusions of the Germ Cells ", Pt. III, ibid., vol. 63, 1918-19.

8. Heidenhain.—" Neue Untersuchungen über die Centralkörper und ihre Beziehungen zum Kern v. Zellenprotoplasma ", ' Arch. f. Mikr. Anat.', vol. 43, 1894.

9. Montgomery (Jr.).—" Comparative Cytological Studies with special regard to the Nucleolus ", ' Journ. of Morph.', vol. 15, 1899.

10. Prenant, Bouin et Maillard.—' Traité d'Histologie ', t. 1.

11. Rio Hortega.—" Investigations sur le tissu musculaire lisse ", ' Trab. del Lab. Invest. Biol. Madrid ', vol. 11, 1913.

12. Vigier.—" Les Pyrénosomes dans les cellules de la glande digestive de l'Écrevisse ", ' Comptes rendus de l'Assoc. des Anatomistes ', nos. 3-4, 1901.

14. EXPLANATION OF PLATES 23 AND 24.

Illustrating Champy and Carleton's paper on ' Observations on the Shape of the Nucleus and its Determination '.

High-power figures drawn at various magnifications.

Arrows point towards the distal (i. e. unattached) ends of the cells.

Lettering.

BC., bile canaliculus ; *BM.*, basement membrane ; *C.*, centrosome ; *C POST.*, posterior centrosome ; *H CAN.*, Holmgren canaliculi ; *LG.*, lipoid granules ; *MI.*, mitochondria ; *MK.*, membrane of Krause ; *NC.*, nuclear canal ; *NI.*, nuclear incision ; *NR.*, nuclear rodlet ; *NU.*, nucleolus ; *PNF.*, peri-nuclear fold ; *SB.*, striated border ; *TN.*, tonofibrillae ; *TS.*, transverse section ; *X.*, invagination of nuclear membrane.

PLATE 23.

Fig. 1.—Showing nuclear incisions in a connective-tissue cell from the Testis of A s t a c u s .

Fig. 2.—Oocytes of the fish S i l u r u s sp., showing how the nucleus becomes polymorphic at a later stage of development.

Fig. 3.—Spermatogonium of B o m b i n a t o r i g n e u s , illustrating that the relation of nucleoli to nuclear folds is not constant. Here the nucleus has many incisions and yet the nucleoli bear but little relation to them.

Fig. 4.—Tubule cell from a nephridium of A u l o s t o m u m—a leech. Folds in nuclear membrane orientated in relation to flattening out of nucleus.

Fig. 5.—Two leucocytes from the lymphoid layer of the liver of the A x o l o t l . In A the nucleus is oval and its membrane highly pleated. B shows a polymorphonuclear white cell derived from A by the partial unfolding of the nuclear incisions. Technique : carbol-formalin and ferric Brazilin.

Fig. 6.—Cell from the hepato-pancreas of O n i s c u s (an Isopod Crustacean) showing deformation of the nucleus by large lipoidal granules in the cytoplasm. Technique : Benda fixation and iron haematoxylin.

Fig. 7.—Human cardiac muscle cells. A is a longitudinal section showing (i) the pleating of the nuclear membrane, each incision corresponding to a membrane of Krause, and (ii) the square ends of the nucleus. B illustrates the arrangement of the nuclear incisions in transverse section at a higher magnification. At the blind end of each incision there is usually a nucleolus. C is a longitudinal and somewhat oblique section of the nucleus, showing the relation of its shape to the fibrils. Technique : carbol-formalin and iron haematoxylin.

Fig. 8.—Cells from gastric epithelium of the A x o l o t l . The nuclear membrane shows deep longitudinal incisions. $TS.=$ a transverse section of the nucleus, the relation of the nuclear incisions to the nuclear membrane being clearly shown.

Fig. 9.—Nucleus of cardiac muscle of A s t a c u s , showing relation between nuclear incisions and nucleoli.

Fig. 10.—Longitudinal nuclear folds in germinal epithelium cell of A x o l o t l .

Fig. 11.—Spermatocyte of L i t h o b i u s f o r f i c a t u s—a Myriapod. An example of a nuclear membrane of regular contour in spite of multiple nucleoli

PLATE 24.

Fig. 1.—Spermatid nuclei of B o m b i n a t o r—a toad. Showing the fully formed axial rodlet in A . B and C are different stages in its

formation. D, E, F, and G show its appearance in transverse section. Technique : Bouin and iron haematoxylin.

Fig. 2.—Nuclei of smooth muscle from the intestine of the Axolotl. A and B are longitudinal sections of nuclei, while C is transverse. All show the spiral circular incisions in the nuclear membrane. Technique : carbol-formalin and iron haematoxylin.

Fig. 3.—Cell from intestine of Oniscus. Note deformation of nucleus by ' Tonofibrillae '.

Fig. 4.—Cell from Wolffian duct of Salamander showing the intra-nuclear canaliculus and centrosomes opposite its aperture.

Fig. 5.—Intestinal epithelial cell from Libellula sp.—a dragon fly. Note that here the length of nucleus is not proportional to that of the cell.

Fig. 6.—Nuclei in longitudinal and transverse section from heart of Helix pomatia (snail). Incisions in nuclear membrane. Technique : Flemming and iron haematoxylin.

Fig. 7.—Hepatic cells of Salamander. At x the nucleolus is in contact with the nuclear membrane, which is slightly invaginated at this point. Technique : Bouin and iron haematoxylin.
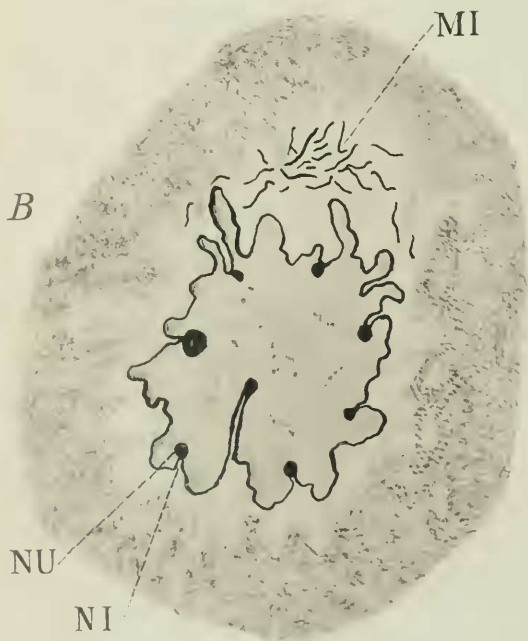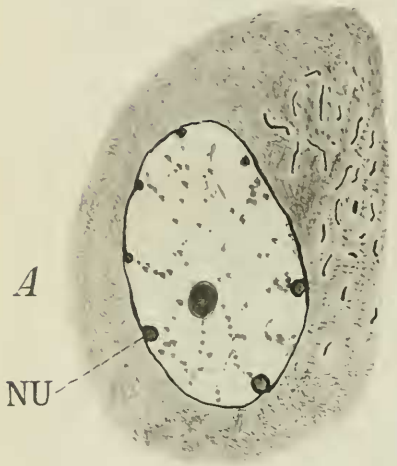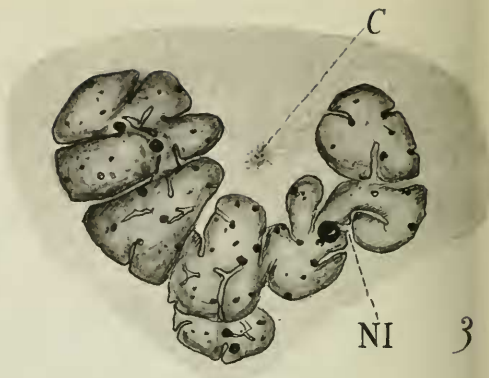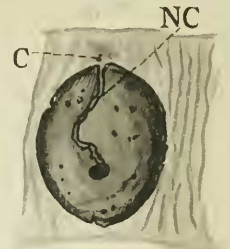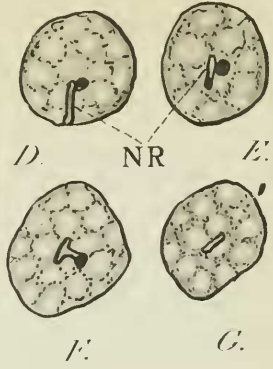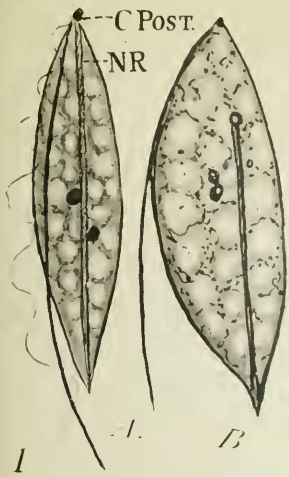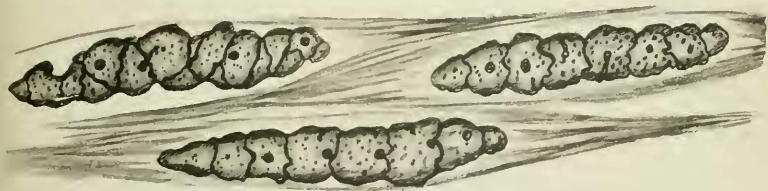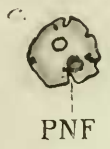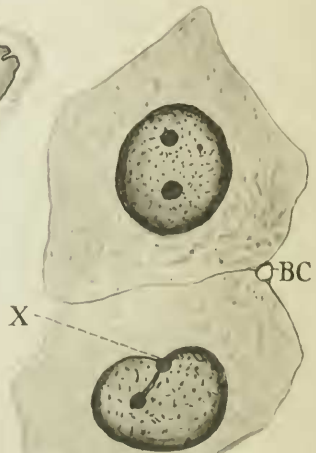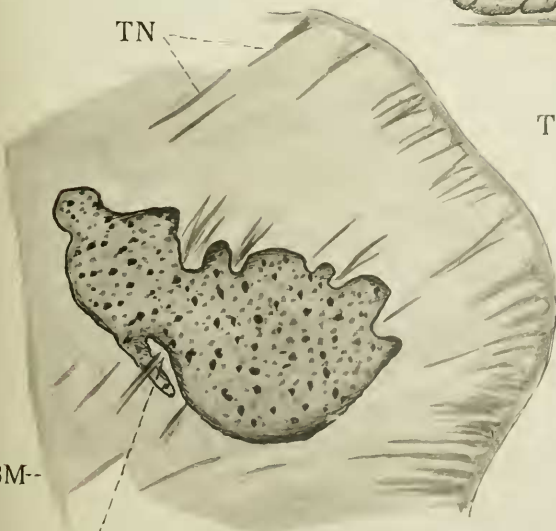
Champy & Carleton
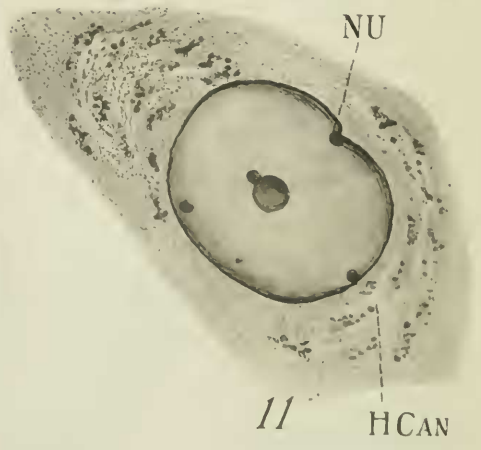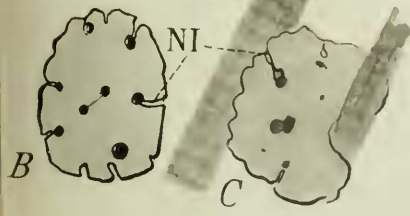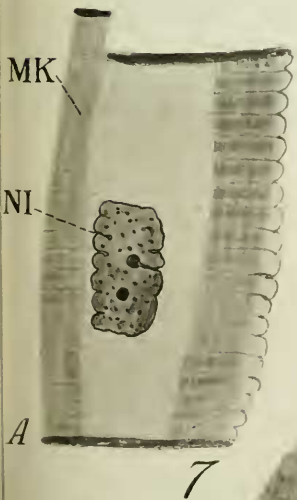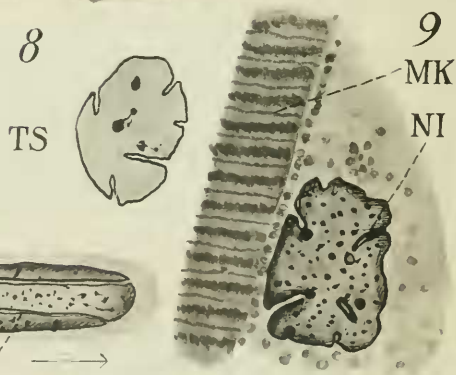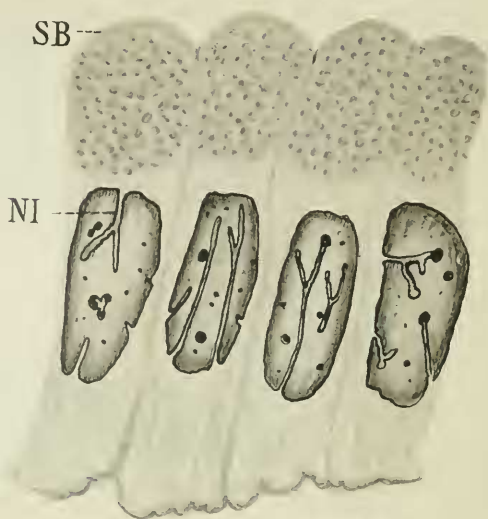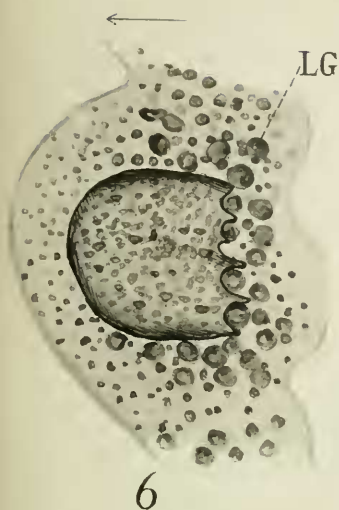
On the calcium carbonate and the calcospherites in the Malpighian tubes and the fat body of Dipterous larvae and the ecdysial elimination of these products of excretion.

By

D. Keilin, Sc.D.,

Beit Memorial Research Fellow.
(From the Quick Laboratory, University of Cambridge.)

With 5 Text-figures.

CONTENTS.

## 1. THE PRESENCE OF CALCIUM CARBONATE IN THE MALPIGHIAN TUBES.

LYONET (**11**, 1832) was the first to notice in the larva of Ptychoptera two milky-white vessels running throughout the length of the body. Similar vessels have been discovered in the larva of Eristalis by Batelli (**1**, 1879), who has rightly described them as saccate dilatations of the anterior pair of Malpighian tubes filled with calcium carbonate. Quite

independently Valery Mayet (**13**, 1896) has shown that in
Cerambyx larvae, of the six Malpighian tubes, four are
larger and are filled with calcium carbonate. The excretion
of this product, which was described by Valery Mayet as a new
function of the Malpighian tubes, was denied by Künckel
d'Herculais (**10**, 1896), who at a meeting of the Entomological
Society of Paris made an observation that Valery Mayet
probably misunderstood the anatomy of the larva, and that
the organs containing calcium carbonate were not Malpighian
tubes but the intestinal caeca. Later, Valery Mayet (**14**, 1896)
succeeded in demonstrating that the tubes in question were
actually the Malpighian tubes ; Künckel d'Herculais then
suggested that the calcium carbonate of Cerambyx larvae
is probably formed in other special glandular cells, and that
the Malpighian tubes were eliminating only the excess of this
product. P. Marchall (**12**, 1896), who took part in this discus-
sion, observed that the excretion of $CaCO_3$ by the Malpighian
tubes has nothing surprising in it ; he thought, however, that
the excretory function in insects is not localized in one particular
organ : uric acid, for instance, can be found not only in the
Malpighian tubes but in the intestine and the fat body.

Calcium carbonate has been found also by Vaney (**19**, 1900 ;
20, 1902) in the anterior pair of the Malpighian tubes of the
Stratiomys larva, and by Pantel (**16**, 1898) in the parasitic
larvae of Tachinidae and in the larvae of Ptychoptera
(**17**, 1914). In the latter, two of the five Malpighian tubes
are transformed into large sacs filled with calcium carbonate.

I myself have found the excreted calcium carbonate in the
Malpighian tubes of many Dipterous larvae : Eristalis
tenax, L., Myiatropa florea, L., Mallota crista-
loides, Lw., Merodon equestris, F., Syritta
pipiens, L., Eumerus strigatus, Fln., Ptycho-
ptera contaminata, L., several species of Stratiomyidae
belonging to the genera Stratiomys, Sargus, and
Odontomyia, and among the Trypetidae in Anastrepha
striata, Schiner. In all of these larvae the carbonate-
containing Malpighian tubes differ from the rest by being

TEXT-FIG. 1.
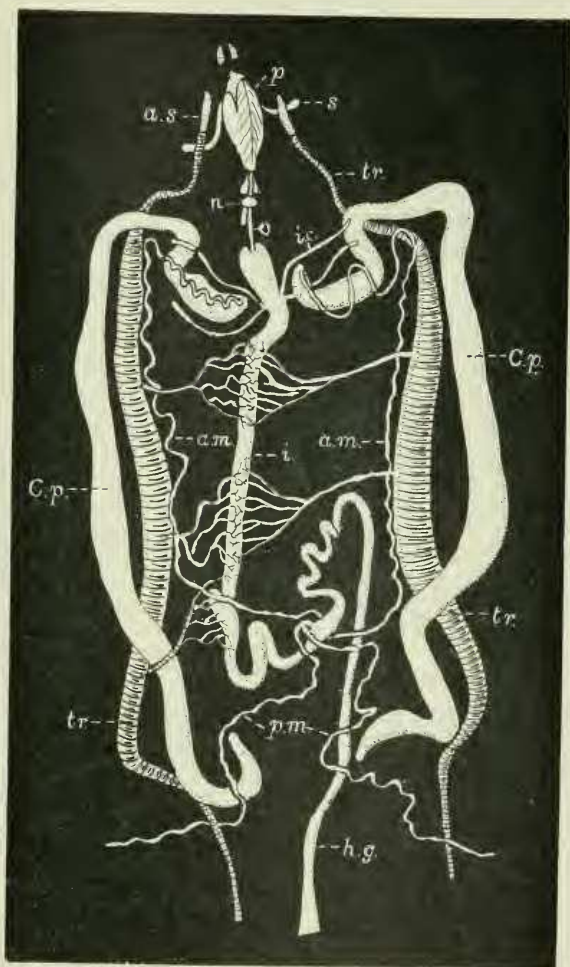
Myiatropa florea, dissection of a full-grown larva. *a.m.*, anterior pair of Malpighian tubes ; *a.s.*, anterior spiracles ; *c.p.*, calcareous or terminal portion of the Malpighian tubes ; *h.g.*, hindgut ; *i.*, mid-gut ; *i.c.*, intestinal caeca ; *n.*, central nervous system ; *o.*, oesophagus ; *p.*, pharynx ; *p.m.*, posterior pair of Malpighian tubes ; *s.*, salivary glands ; *tr.*, tracheal trunks.

more developed and of a milky colour. In the larva of
Ptychoptera contaminata and of a few Eristalids,
these tubes, at least in their terminal portions, are excep-
tionally well developed and can be easily seen by transparency
with the naked eye. Text-fig. 1, which represents a complete
dissection of the larva of Myiatropa florea, L., shows
to what extent the calcareous portion of the Malpighian tube
can be developed in a full-grown larva. In this example the
posterior pair of Malpighian tubes (*p.m.*) is composed of two
short branches of normal structure ; the anterior pair (*a.m.*),
on the contrary, is very long, its two branches in their proximal
portion are of normal structure and diameter and extend to
the anterior portion of the body, where they suddenly pass
into two enormous sacs (*c.p.*) with milky contents, which run
backwards and reach posteriorly the anal segment. These two
sacs are even thicker than the intestine of the larva ; they are
very fragile, and the slightest puncture causes their milky
contents to flood out. The milky fluid is composed of a thick
suspension of very small calcareous granules which are almost
completely soluble in dilute acid, only a small central particle,
probably of an organic nature, remaining.

2. Calcospherites in the Fat Body.

In all of the above-mentioned larvae the calcium carbonate
of the Malpighian tubes appears in the form of crowded small
granules suspended in the fluid which fills the lumen of these
tubes. There are, however, other larvae which contain the
calcium carbonate in form of calcospherites. The
latter are enclosed either in the anterior pair of the Malpighian
tubes or in special cells connected with the fat body.

The term calcospherite we owe to Harting (**7,** 1873), who
was the first to prepare, artificially, calcareous corpuscles
composed of two substances, mineral and organic.
He obtained these bodies by precipitating calcium carbonate
($CaCl_2 + K_2CO_3 = CaCo_3 + 2KCl$) in a liquid containing organic
matter (albumen, for instance). The calcareous corpuscles
thus obtained were elongated or spherical, highly refractive,

composed of numerous concentric layers surrounding a central or excentric granulated body and bearing some resemblance to starch grains. When the calcospherites are dissolved in dilute acetic acid there remains an albuminoid stroma consisting of calcoglobulin. Examined in polarized light, the calcospherites show a black cross. The calcospherites, or Harting's corpuscles, have been well described by Nathusius (**15**, 1890), who found them in numerous animals and plants, and by Pettit (**18**, 1897) in cases of pathological ossification in mammals.[1]

In insects the calcospherites were discovered simultaneously by Henneguy (**8**, 1897) and Giard (unpublished observations quoted by Henneguy). Henneguy found them in the larvae of Phytomyza chrysanthemi, Kowarz. According to this author each calcospherite of this larva is enclosed in a special hypertrophied cell of the fat body. The fat of these cells disappears completely, and all that remains of the cell is reduced to a thin protoplasmic layer and a small degenerated nucleus. The calcospherites still appear in the pupa, but they are absent in the adult flies, and Henneguy thought that the imagines which he examined were probably obtained from the ' normal ' larvae, i. e. ' larvae devoid of calcospherites '. Giard has observed similar calcospherites in the larvae of Phytomyza lateralis, Fall., which attacks the inflorescence of Matricaria inodora.

Personally I have found the calcospherites in the fat body of many Phytomyzine and Agromyzine larvae (Text-fig. 2). In all the species where the calcospherites are present they are to be found in every individual larva throughout its life. In this my observations differ from those of Henneguy and Giard, who considered the presence of calcospherites as abnormal and probably only seasonal. The cells which contain the calcospherites are always connected with the fat body, although they never contain droplets of fat. As a rule they lie in alveolar spaces formed among the fat cells (Text-fig. 3).

[1] To these two papers the reader is referred for numerous observations and references concerning this subject.
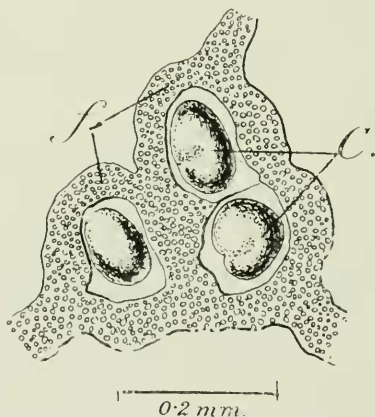
The calcospherites are already present in very young larvae, but not in the embryo or in those just hatched ; they seem to

TEXT-FIG. 2.

1 mm.
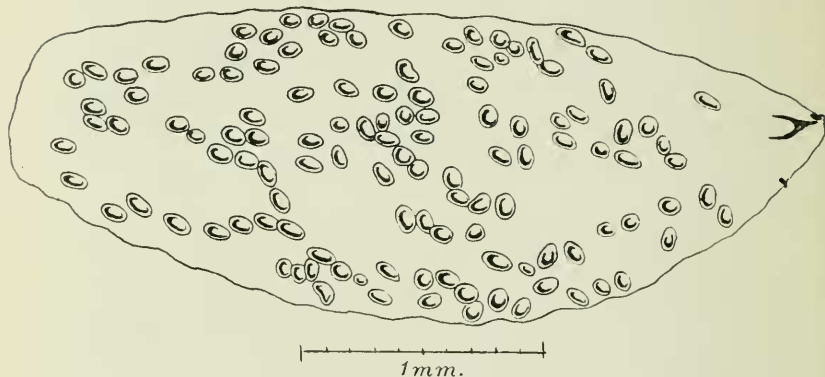
A g r o m y z a sp., full-grown larva, slightly compressed, showing by transparency 120 calcospherites disseminated throughout the body.

TEXT-FIG. 3.

0·2 m m.

A g r o m y z a larva, a portion of the fat body, f., showing the calcospherites, c.

appear only after a short period of feeding. The existence of calcospherites in larvae belonging to the families Phytomy-zinae and Agromyzinae seems to be so general that this character

assumes a taxonomic importance and helps one to recognize these larvae and to differentiate them from the phytophagous larvae belonging to other families like Anthomyidae and Trypetidae, the fat body of which is devoid of calcospherite cells.
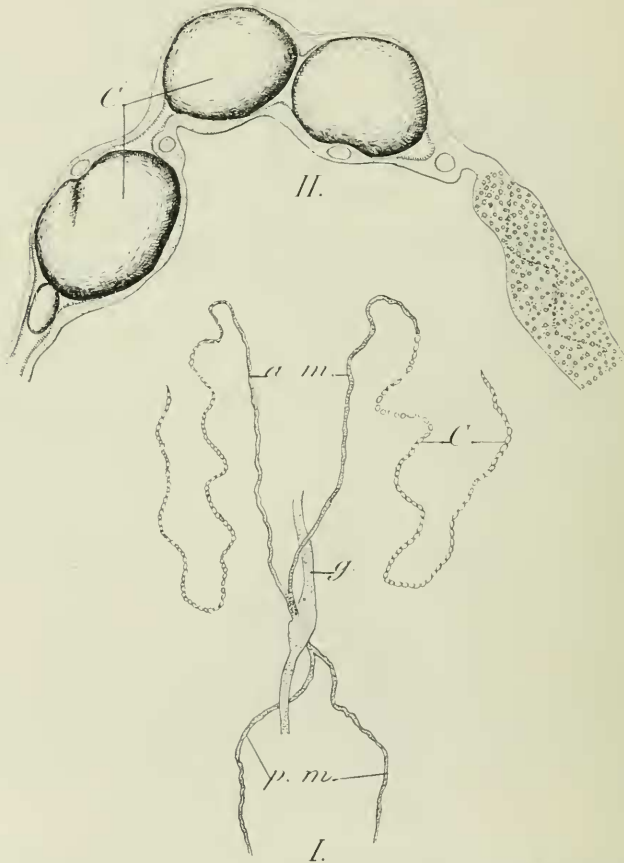
3. Calcospherites in the Malpighian Tubes.

The only case of the existence of calcospherites in the Malpighian tubes is that of the larva of Acidia heraclei, the celery-fly larva. On examining a living larva of Acidia gently compressed between the slide and coverglass, I have noticed that its body contains a number of large calcospherites similar to those of Agromyzine larva. I thought at first that the calcospherites of Acidia larvae were also formed in special cells connected with the fat body. The dissection of these larvae revealed that such was not the case; all the calcospherites were lying free in the lumen of the Malpighian tubes and especially in their terminal portions (Text-fig. 4, I and II, c.). The calcospherites of various sizes, from $8\,\mu$ to $140\,\mu$ in diameter, distend these tubes, which have the appearance of being composed of highly refractive beads. The calcospherites when small are very often double, i. e. with two or more central granules (Text-fig. 5, b, c, and d). The occurrence of the calcospherites in the Malpighian tubes (Acidia heraclei) and in the fat body (Agromyzinae) of the phytophagous Dipterous larvae demonstrates once more the similarity in the excretory function of these two larval organs.

4. Ecdysial Elimination of Calcium Carbonate during Metamorphosis.

All the foregoing shows that the larvae of a great number of Diptera contain in their Malpighian tubes, or in the cells connected with the fat body, a large quantity of calcium carbonate stored in the form of minute granules or large calcospherites.

A question now arises : What becomes of the stored calcium carbonate during the ultimate stages of the life of the insect ?

TEXT-FIG. 4.

Acidia heraclei. I. *a.m.*, anterior pair of the Malpighian tubes ; *c.*, terminal portion filled with the calcospherites ; *g.*, gut ; *p.m.*, posterior pair of Malpighian tubes. II. portion of the anterior pair of Malpighian tubes showing the calcospherites free in the lumen of the tube.

According to Pantel (**17**, 1914) the calcium carbonate of the Ptychoptera larva disappears before the metamorphosis takes place. He considered that it does not dissolve

in the body of the larva, but passes from the Malpighian tubes into the hind-gut, whence it is expelled from the body just before the larva begins to pupate. He admits, however, that he never actually saw the process of expulsion of this product

TEXT-FIG. 5.
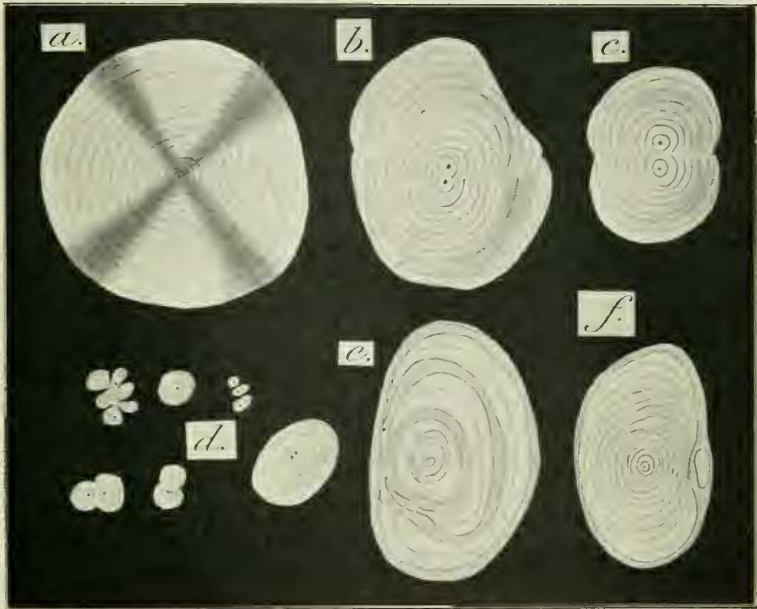
Calcospherites of Acidia heraclei. *a* to *e*. *a*, a calcospherite examined by polarized light, showing the black cross; *b* and *c*, double calcospherites; *d*, very small simple, double or multiple calcospherites; *e*, calcospherite in diluted acetic acid showing collapsing stroma; *f*, intracellular calcospherite of Agromyza larva.

of excretion. In several cases he found the calcareous substance retained in the pupae of Ptychoptera.

Henneguy (**8,** 1897) found that the calcospherites of Agromyza larvae persist in the pupae, but he did not find them in the adult flies. He considered that the existence of calcospherites was not general, and was very probably abnormal,